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Determination of the frequency and distribution of vascular and parenchymal amyloid with polyclonal and N-terminal-specific PrP antibodies in scrapie-affected sheep and mice.

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Abstract

Brains from 17 histopathologically confirmed cases of scrapie, five of which had congophilic vascular amyloid, were stained immunohistochemically for prion protein (PrP) using a polyclonal antibody. Two clinically suspect but pathologically unconfirmed cases of natural sheep scrapie and the brains of four mice infected with the 111A murine scrapie strain were also examined. Selected sections containing amyloid were stained with each of two peptide antibodies which recognise the N-terminal amino acid residues which are lost following protease digestion of the disease-specific isoform of PrP. The mice infected with the 111A murine scrapie strain had large numbers of hypermature plaques. All the amyloid plaques from both natural sheep scrapie brains and experimental murine brains were heavily immunostained by the polyclonal and both peptide antibodies. In addition, disease-specific accumulations of PrP were detected in endothelial cells or in the intima of blood vessels of the cerebral cortex of sheep scrapie brains. The affected blood vessels were located in areas which otherwise lacked typical scrapie pathology. Vascular accumulations of PrP were also found in leptomeningeal and choroid plexus blood vessels. Vascular amyloid was found mainly in the neocortex. Vascular amyloid and disease-specific parenchymal accumulations of PrP were found in two sheep which showed clinical signs of scrapie but lacked its typical vacuolar pathology. These results show that the mature amyloid of scrapie is composed of, or contains a substantial proportion of, whole length PrP protein. Thus truncation of PrP is not essential for the aggregation of PrP into amyloid. The vascular amyloid of natural sheep scrapie originates from the accumulation and release of PrP from endothelial cells presumably following systemic scrapie infection. The topography of vascular amyloid distribution in Great Britain differs from that reported in the Netherlands. As amyloid deposition in mice is largely controlled by the strain of the infecting agent it is possible that the strain of the agent may influence vascular amyloid deposition.

MeSH

16: 1141-1155 (1988)). The procedure may involve immobilized sequence-specific oligonucleotides probes (Saiki et al, supra).

5.2 DETECTING THE CF 507 MUTATION

These detection methods may be applied to prenatal diagnosis using amniotic fluid cells, chorionic villi biopsy or sorting fetal cells from maternal circulation. The test for CF carriers in the population may be incorporated as an essential component in a broad-scale genetic testing program for common diseases.

According to an embodiment of the invention, the portion of the DNA segment that is informative for a mutation, such as the mutation according to this embodiment, that is, the portion that immediately surrounds the I507 deletion, can then be amplified by using standard PCR techniques [as reviewed in Landegren, Ulf, Robert Kaiser, C. Thomas Caskey, and Leroy Hood, DNA Diagnostics - Molecular Techniques and Automation, in Science 242: 229-237 (1988)]. It is contemplated that the portion of the DNA segment which is used may be a single DNA segment or a mixture of different DNA segments. A detailed description of this technique now follows.

A specific region of genomic DNA from the person or fetus is to be screened. Such specific region is defined by the oligonucleotide primers C16B (5'GTTTTCCTGGATTATGCCTGGCAC3') and C16D (5'GTTGGCATGCTTTGATGACGCTTC3') or as shown in Figure 18 by primers 10i-5 and 10i-3. The specific regions using 10i-5 and 10i-3 were amplified by the polymerase chain reaction (PCR). 200-400 ng of genomic DNA, from either cultured lymphoblasts or peripheral blood samples of CF individuals and their parents, were used in each PCR with the oligonucleotides primers indicated above. The oligonucleotides were purified with Oligonucleotide Purification Cartridges™ (Applied Biosystems) or NENSORB™ PREP columns (Dupont) with procedures recommended by the